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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/700,291	11/03/2003	David J. Wasilko	PC23192A	6793

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PFIZER INC.
PATENT DEPARTMENT, MS8260-1611
EASTERN POINT ROAD
GROTON, CT 06340

EXAMINER	
CHEN, STACY BROWN	

ART UNIT	PAPER NUMBER
1648	

DATE MAILED: 05/10/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/700,291	WASILKO ET AL.	
	Examiner	Art Unit	
	Stacy B. Chen	1648	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *31 January 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-83* is/are pending in the application.

4a) Of the above claim(s) *32-36 and 57-83* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-31 and 37-56* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *03 November 2003* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11/03, 6/04, 3/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election with traverse of Group I, filed January 31, 2005 is acknowledged. Claims 1-83 are pending. It is noted that the election/restriction requirement indicated that claim 56 was included in Group I, however, according to the subject matter of claim 56, it belongs in Group III, a method of infecting cells. The examiner mistakenly included claim 56 in Groups I and III. Therefore, Claims 32-36 and 56-83 are withdrawn from consideration, being drawn to non-elected subject matter. Claims 1-31 and 37-55 are under examination. Applicant's traversal of the restriction requirement has been carefully considered but fails to persuade. Applicant mainly argues that the Restriction Requirement has not demonstrated a serious burden to examine all of the claims. In response, the Office has established that there would be serious burden to examine all claims. (See the Restriction Requirement of December 28, 2004, page 4, first paragraph.) A search for the viruses of Group II does not require a search of the method of producing the viruses, as claimed in the claims of Group II, product by process. A search of the literature for viruses and host cells will not necessarily reveal composition of viruses and host cells in combination with a cryoprotective agent. Therefore, the restriction requirement is deemed proper and made FINAL.

Information Disclosure Statement

2. In the IDS filed November 3, 2003, the US Patent was not considered because the citation is incorrect. Applicant is invited to correct this citation.

Claim Rejections - 35 USC § 102/103

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-7, 9-13, 15-18, 21, 37-42, 44-46, 49, 50 and 52-54 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Reimann *et al.* (*Clinical and Diagnostic Laboratory Immunology*, 2000, 7(3):352-359, herein, "Reimann"). The claims are drawn to a cryogenically protected viral delivery system for infecting host cells comprising a cryogenic vessel and a plurality of virally infected cells in admixture with a cryo-protective agent contained in the cryogenic vessel at a temperature of less than or equal to -20°C. The concentration of virally infected cells is from 10^6 cells/ml to 10^9 cells/ml, and the viability of cells contained in the vessel is at least 50%. The vessel contains 10^5 to 10^{12} cells. The viability is at least 70% or at least 90%, and the temperature of the admixture is less than or equal to -70°C, or -130°C. The admixture is substantially free of extracellular viral particles and spend incubation media. The average cell diameter of cells contained in the cryogenic vessel is at least 0.5 microns greater than the average cell diameter of uninfected cells of the same type. The cryo-protective agent is DMSO. The volume of the vessel is less than or

equal to 250 ml, 30 ml, or 6 ml. The virally infected cells represent at least 20%, 40% or 60% of the total number of cells in the vessel. The virally infected cells are eukaryotic cells infected with a virus, such as HIV-1. Also claimed is a method of preparing a cryogenically protected viral delivery system comprising admixing a plurality of virally infected cells with a cryo-protective agent to obtain an admixture having a concentration of virally infected cells of from 10^6 cells/ml to 10^9 cells/ml, and freezing at least a portion of the admixture for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -20°C and the viability of the cells in the frozen admixture is at least 50%. The temperature is reduced at a rate from 1°C/minute to 30°C/minute. The admixture is aliquoted into the cryogenic vessels in an amount from 10^8 to 10^9 virally infected cells. The volume of the admixture aliquoted into the vessels is from 0.5 ml to 20 ml.

Reimann discloses the cryopreservation of peripheral mononuclear blood cells (PMBCs) from HIV-1 infected donors. On page 353, column 1, *Materials and Methods* section, Cryopreservation and thawing of PBMC, the method of cryopreservation and measurement of viability is described. Infected PBMCs were isolated from blood and resuspended in fetal bovine serum with 10% dimethyl sulfoxide (DMSO) at 10^7 cells/ml. The aliquots into the cryovials were 0.5-1.0 ml. The filled cryovials were then placed in a 4°C freezing container and placed in a -70°C freezer overnight. Given that the cryovials were placed into the -70°C freezer, the temperature of the contents of the vial changed from 0°C to -70°C. At some point of this initial freeze, the rate of freezing is expected to be from 1°C/minute to 30°C/minute. After the samples were frozen, they were transferred to a liquid nitrogen freezer (-70°C). Viability of the HIV-1infected PBMCs was measured via trypan blue dye exclusion. The viability of the PBMCs

prior to freezing was 98%, and the viability post-thawing was 95% (page 352, column 1, Results section, *Specimen donors and PBMC viability*). Regarding the claim limitations of the admixture being substantially free of extracellular viral particles and spend incubation media, Reimann discloses that the PBMCs in blood were initially stored (incubated) with EDTA or heparin, followed by density gradient centrifugation, lysis of contaminating red blood cells and washing. One would expect that Reimann's protocol removed extracellular viral particles and spent incubation media (EDTA or heparin which the PBMCs were stored in). Further, regarding the limitation of the virally infected cell diameter being at least 0.5 microns greater than the average cell diameter of uninfected cells of the same type, one would expect that a cell that is infected with viruses would be at least 0.5 microns greater in diameter. Given that more than one virus infected Reimann's PBMCs, and the size of viruses being no larger than 0.3 microns generally, one would expect that the infection of cells with multiple viruses and their progeny would increase the diameter of the cell by at least 0.5 microns. Therefore, the claims are anticipated by, or in the alternative, obvious over Reimann.

4. Claims 14 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Reimann as applied to claims 1-7, 9-13, 15-18, 21, 37-42, 44-46, 49, 50 and 52-54 above, and further in view of Wisniewski (US Patent 6,337,205). The claims are drawn to a cryogenically protected viral delivery system for infecting host cells comprising a cryogenic vessel and a plurality of virally infected cells in admixture with a cryo-protective agent contained in the cryogenic vessel at a temperature of less than or equal to -20°C. The vessel is a polypropylene vial, more specifically, a polypropylene vial having a volume of less than or equal to 6 ml. Also

claimed is a method of preparing a cryogenically protected viral delivery system comprising admixing a plurality of virally infected cells with a cryo-protective agent to obtain an admixture having a concentration of virally infected cells of from 10^6 cells/ml to 10^9 cells/ml, and freezing at least a portion of the admixture for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -20°C. The teachings of Reimann are summarized above. Reimann is silent on the specific type of cryovial that was employed, however, Wisniewski discloses a method of cryopreservation of biological materials, including cells and viruses, comprising packing the biologicals in a polypropylene cryovial containing DMSO (claim 1, col. 13, lines 8-13 and 32-49, and col. 6, lines 65-67). It would have been obvious to one of ordinary skill in the art to use the polypropylene vials taught by Wisniewski in Reimann's method. One would have been motivated to use polypropylene vials because Wisniewski teaches that polypropylene polymers are a cryogenically stable material compatible with biopharamaceutical products and cryopreservation fluid in liquid and frozen states (col. 6, lines 65-67). One would have had a reasonable expectation of success that polypropylene vials would have successfully served as suitable vials for Reimann's cryopreservation method because Wisniewski uses polypropylene vials for cryopreserving cells, viruses, and other biological products. Therefore, the invention of claims 14 and 43 would have been obvious to one of ordinary skill in the art at the time of the invention.

5. Claims 1-31 and 37-55 rejected under 35 U.S.C. 103(a) as being unpatentable over Witt *et al.* (*Journal of Virological Methods*, 1987, 17:287-292, herein, "Witt") in view of Freshney (*Cryopreservation*, Chapter 19, Culture of Animal Cells: a manual of basic technique, 4th edition,

Wiley-Liss, 2000, pages 297-308), Invitrogen™'s *Guide to Baculovirus Expression Vector Systems and Insect Cell Culture Techniques*, Kistner *et al.* (*Vaccine*, 1998, 16(9/10):960-968, herein, "Kistner"), Clontech Laboratories, Inc. document (Protocol #PT3494-2, Version #PR19432, published in 2001, 2 pages, available at http://www.clontech.com) and Nienhuis (US Patent 5,780,447). The claims are drawn to a cryogenically protected viral delivery system for infecting host cells comprising a cryogenic vessel and a plurality of virally infected cells in admixture with a cryo-protective agent contained in the cryogenic vessel at a temperature of less than or equal to -20°C. The concentration of virally infected cells is from 10^6 cells/ml to 10^9 cells/ml, and the viability of cells contained in the vessel is at least 50%. The vessel contains 10^5 to 10^{12} cells. The viability is at least 70% or at least 90%, and the temperature of the admixture is less than or equal to -70°C, or -130°C. The admixture is substantially free of extracellular viral particles and spend incubation media. The average cell diameter of cells contained in the cryogenic vessel is at least 0.5 microns greater than the average cell diameter of uninfected cells of the same type. The cryo-protective agent is DMSO. The volume of the vessel is less than or equal to 250 ml, 30 ml, or 6 ml. The vessel is a polypropylene vial having a volume of less than or equal to 6 ml. The virally infected cells represent at least 20%, 40% or 60% of the total number of cells in the vessel. The virally infected cells are eukaryotic cells infected with a virus or recombinant virus, baculovirus, adenovirus, influenza or adeno-associated virus. The virally infected cells can also be insect cells, such as Sf9 infected with baculovirus. The Sf9 cells infected with recombinant baculovirus carry a heterologous polynucleotide operatively linked to a baculovirus polyhedrin promoter. The cells are mammalian, such as HEK-293 cells. Also claimed is a method of preparing a cryogenically protected viral delivery system comprising

admixing a plurality of virally infected cells with a cryo-protective agent to obtain an admixture having a concentration of virally infected cells of from 10^6 cells/ml to 10^9 cells/ml, and freezing at least a portion of the admixture for a time and under conditions sufficient so that the temperature of the frozen admixture is less than or equal to -20°C and the viability of the cells in the frozen admixture is at least 50%. The temperature is reduced at a rate from 1°C/minute to 30°C/minute. The admixture is aliquoted into the cryogenic vessels in an amount from 10^8 to 10^9 virally infected cells. The volume of the admixture aliquoted into the vessels is from 0.5 ml to 20 ml.

Witt discloses the cryogenic preservation of virus-infected cells used as immunofluorescent assay substrates. Witt demonstrates that virus-infected cells can be frozen, thawed, and used for further purposes (page 290, last paragraph). The cell lines used were the mammalian cells VERO, HeLa and HEp-2 cells. The cell lines were grown in media and infected with adenovirus 5 (Ad5), herpes simplex virus (HSV) and respiratory syncytial virus (RSV). The infected cells demonstrating cytopathic effect were sedimented, washed with DPBS, resuspended into equal aliquots and sedimented again, and resuspended in freezing media that included DMSO. The cells were placed in different freezers at -20°C and -70°C, whose temperatures were lowered by a controlled freezing rate down to -165°C (page 289, entire page of methods). Witt discloses that the infected cells retained their structural integrity and antigenicity following thawing and subsequent processing (page 290, first paragraph). Witt is silent on the amount of virally infected cells, influenza, baculovirus, HEK-293 cells and Sf9 insect cells.

However, Freshney discloses general techniques of mammalian cell culture that are applicable to the virus-infected cells of Witt. Specifically, Freshney teaches a general protocol for freezing cells that includes culturing a high-cell density suspension, centrifuging/washing, adding 5-10% DMSO or 10-15% glycerol at approximately 10^6 to 10^7 cells/ml. Freshney teaches that cell suspensions at a high concentration should be frozen at approximately 1°C per minute (page 298, second column, first full paragraph). It would have been obvious to incorporate Freshney's teachings in the method of Witt because Freshney's methods are applicable to mammalian cell cultures. One would have been motivated to use Freshney's teachings on cryopreservation in Witt's method because Freshney teaches that freezing cells reduces risks of genotypic drift, senescence, transformation, phenotypic instability, contamination and incubator failure, for example (page 297).

Invitrogen™'s *Guide to Baculovirus Expression Vector Systems and Insect Cell Culture Techniques* provides teachings on the expression capabilities of recombinant baculovirus cultured in insect cells (page 1). Protocol 15 on page 16 details the culture, infection and storage of insect cells infected with recombinant baculovirus. Insect cells are Sf9 (Table 1) that comprise a heterologous nucleic acid sequence and polyhedrin promoter (page 3, column 1, section, "Generating a Recombinant Virus by Homologous Recombination"). Invitrogen™'s protocol (Protocol 16) includes culturing insect cells and allowing cell density to reach about 10^6 cells/ml, followed by infection with baculovirus, pelleting of cells and storing the pellet at -20°C. The protocol uses polypropylene cryovials (Protocol 15, page 16).

Kistner discloses influenza infected Vero cells used for propagating influenza virus for vaccine purposes (abstract). Clontech Laboratories, Inc. document (Protocol #PT3494-2,

Version #PR19432, published in 2001, 2 pages, available at http://www.clontech.com) teaches that HEK-293 cells are used for recombinant adenovirus expression systems (page 1, first paragraph). Nienhuis (US Patent 5,780,447) discloses that adeno-associated viral vectors are useful for gene transfer and expression of desired proteins in cells (col. 3, lines 21-27).

It would have been obvious to use Witt's method to freeze any of the virus-infected cells of baculovirus in Sf9 cells, influenza in Vero cells, and adeno-associated virus in HEK-293 cells. One would have been motivated to express any of these virus-infected cells in Witt's method because Witt teaches that inventories of infected cells could be maintained, thus eliminating repetitive culturing, if virally infected cell lines are preserved (Witt, page 288, second full paragraph). One would have had a reasonable expectation of success that these virus-infected cells would have been frozen because Witt successfully froze RSV, HSV and Adenovirus-infected cells. While the references cited do not explicitly recite the percent viability of the infected cells, one would have expected the cells to have at least 50% viability because the methods of cryopreservation required steps of determining viability prior to freezing and post-freezing. With regard to the limitation about the collection of virally infected cells in a vessel having a size of at least 100 ml, none of the above-cited reference details the volume of the collection receptacle. However, the culture of cells involves media that must be present in an amount sufficient to accommodate the cells' nutritional needs. The scale-up of any cell culture or viral production process is well within the abilities of one of ordinary skill. Further, the collection receptacle is an intermediary step and does not render the final product or the process patentable. The volume of aliquots, the volume of the cryovials, the cell viability and percent of virally infected cells are all variables of cryopreservation that one of ordinary skill would know

how to tailor and optimize. Although Applicant asserts that a method of cryopreservation of virally infected cells is novel and unobvious, the prior art shows that virally infected cells have been frozen at low temperatures and retained their structure and antigenicity due to the freezing solution, among other variables. Therefore, the invention as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

6. No claim is allowed. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stacy B. Chen whose telephone number is 571-272-0896. The examiner can normally be reached on M-F (7:00-4:30). If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James C. Housel can be reached on 571-272-0902. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Stacy B. Chen
April 15, 2005

James C. Housel 4/16/05

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